BMC Infectious Diseases

RESEARCH

Open Access

Impact of hepatitis C virus genotype on the efficacy of the direct-acting antivirals in chronic kidney disease patients in West Bengal, India

Sagnik Bakshi[1†], Supradip Dutta[1†], Aritra Biswas[1], Raina Das[1], Shreyasi Nath[1], Anwesha Ghosh[1], Upasana Baskey[1] and Provash Chandra Sadhukhan[1*]

Abstract

Background Hepatitis C virus (HCV) infection increases the risk of mortality and morbidity among chronic kidney disease (CKD) patients. However, the advancement of HCV treatment has made this viral infection curable. Thus, the main objective of this study was to comprehend the HCV genotype (GT) distribution and the efficacy of direct-acting antivirals (DAAs) among CKD patients in West Bengal.

Methods Over five years (January 2017 to December 2021), 310 HCV sero-reactive patients were enrolled in this observational prospective study. HCV RNA was quantified using qRT-PCR. The partial amplification of the core (405 bp) and NS5B (389 bp) region was performed by nested RT-PCR followed by Sanger sequencing for HCV genotype analysis using the NCBI genotyping tool. The phylogenetic tree was constructed using the MEGA-X tool.

Results The occurrence of HCV RNA positivity was 50.64% ($n=157$), and of these 157 patients, 141 (89.81%) completed the DAAs treatment. The most important observation of the study was the prevalence of uncommon HCV genotype GT-1c (67.52%) followed by 1a, 4a, 3a, 1b, and 3b among CKD patients. The overall DAAs efficacy between January 2017 and December 2018 was ~97%, and in January 2019 and December 2021, ~95% among CKD patients. At the same time, in these two phases, DAAs efficacy among GT-1c-infected CKD patients was ˜ 96% and ˜ 93%, respectively.

Conclusions The prevalence of GT-1c among CKD patients was unusual in this geographic region. The overall efficacy of DAAs among the CKD population was encouraging. However, the downtrend of the DAAs efficacy in GT-1c may increase concern among this high-risk group in the future.

Clinical trial Not applicable.

Keywords Hepatitis C virus (HCV), Chronic kidney disease (CKD), HCV genotype (GT), Direct-acting antivirals (DAAs)

[†]Sagnik Bakshi and Supradip Dutta contributed equally to this work.

*Correspondence:
Provash Chandra Sadhukhan
sadhukhan.pc@icmr.gov.in; provash2000@gmail.com

[1]Present address: Division of ICMR-NICED Virus Laboratory, Indian Council of Medical Research- National Institute for Research in Bacterial Infections (ICMR- NIRBI, formerly ICMR-NICED), P-33, Scheme XM, CIT Road, Beliaghata, Kolkata, West Bengal 700010, India

Introduction

Kidney Disease Improving Global Outcomes (KDIGO) defines chronic kidney disease (CKD) as reduced kidney function with glomerular filtration rate (GFR) < 60 ml/min per 1.73 m^2 or the upregulation of kidney disease markers or both for at least three months [1]. Age, diabetes mellitus, heart disease, hypertension, serum lipids, obesity, and consumption of alcohol are the key risk factors for developing CKD [2]. Global Burden of Disease (GBD) reported that the prevalence of CKD has increased by 33% between 1990 and 2017, and one-third of CKD patients are living in India and China alone [3]. Several studies reported that CKD patients are prone to acquiring secondary infections like hepatitis C infection due to frequent exposure to direct contact with infected patients, transfusion of contaminated blood products, or contaminated equipment during dialysis. HCV infection quickened the end-stage renal progression and increased the mortality and morbidity rates in CKD patients [4]. Several studies reported that the HCV infection rate in India among the CKD population ranges between 4.3% and 45% [5–7].

HCV is an enveloped, positive-sense RNA virus with a genome size of ~9.6 kb, belonging to the Flaviviridae family and Hepacivirus genus. This virus is associated with the risk of developing chronic liver diseases, including cirrhosis of the liver and end-stage liver diseases like hepatocellular carcinoma (HCC) [8]. World Health Organization (WHO) estimates that there are 58 million HCV infections globally, with around 1.5 million new cases every year and 290,000 HCV-related fatalities [9]. In India, the number of HCV infections is approximately 6–12 million [10]. Till now, HCV genotypes have been classified into eight and 86 subtypes [11]. In recent years, direct-acting antivirals (DAAs) have been used as a gold standard to combat HCV. The introduction of DAAs transforms the treatment of HCV infection, including CKD patients, who are considered a challenging population in therapeutic management [11]. Therefore, it is important to frequently evaluate HCV infection and the efficacy among CKD patients for better treatment of HCV in this high-risk group (HRG). Few studies indicated circulating HCV genotypes in this group from the western, southern, and north-eastern parts of India [12, 13]. However, there is a lack of information on the circulating HCV genotype among CKD patients in the eastern region of India. This study will provide that information. The Government of India (GOI) launched a National Viral Hepatitis Programme (NVHCP) in 2018 to eliminate hepatitis C infection by 2030 [14]. Thus, it is important to know the treatment efficacy of DAAs among CKD patients and the impact of HCV genotype on the treatment outcome in this high-risk group population. A five-year study was conducted to highlight the demographic analysis, viremia rate, HCV genomic diversity, and DAAs treatment efficacy among CKD patients in West Bengal, the eastern region of India.

Materials & methods

Study design

In the five years (January 2017 to December 2021), 310 HCV sero-reactive CKD patients' whole blood samples were collected for this observational prospective study. HCV sero-reactive patients are defined as individuals who have been infected with HCV at a certain point in time. An HCV sero-reactivity screening test was done, using the HCV antibody Enzyme-Linked Immunosorbent Assay (ELISA) test. However, a positive antibody test does not mean the individuals are infected with HCV. Therefore, an additional Nucleic Acid Test (NAT), an RT-PCR test, is required for confirmation that the patient still has HCV infection. So, if a patient is reactive and RT-PCR positive, we consider the patient to have an active HCV infection and they are HCV RNA-positive patients [15]. The patients were referred from different hospitals in West Bengal, India, and samples were collected in ICMR-NIRBI (Formerly ICMR-NICED). The serum was separated and stored at -80°C for further study. Demographic and clinical data of the patients were collected at the time of enrolment and were maintained electronically. The study was divided into two periods based on their treatment regimen. Briefly, those HCV RNA-positive CKD patients enrolled between January 2017 and December 2018 who underwent DAAs treatment received the combination of 400 mg Sofosbuvir (SOF)/ 90 mg Ledipasvir (LDV) or 400 mg SOF/ 60 mg Daclatasvir (DCV) for HCV treatment [16]. Whereas those HCV RNA-positive CKD patients enrolled between January 2019 and December 2021 who underwent DAAs treatment received 400 mg SOF/ 60 mg DCV or 400 mg SOF/ 100 mg Velpatasvir (VEL) as an HCV treatment regimen [14]. We could not enroll CKD patients between March 2020 and June 2020 due to the COVID-19 pandemic. Individuals with proper treatment information and those who have undergone dialysis for more than six months were only included in this study. Patients with ≥ 14 years were included in this study. Patients co-infected with hepatitis A, B, E, or human immunodeficiency virus (HIV) were excluded from this study.

Viral RNA isolation and HCV viral load Estimation

Viral RNA was isolated from 140 µl of HCV sero-reactive serum samples using a QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), eluted in 50 µl elution buffer, and stored at -80° C. The HCV RNA was determined quantitatively based on 5' UTR using Real-time PCR. A Quanti-Fast Pathogen RT-PCR + IC kit (Qiagen, Hilden,Germany) was used to determine HCV viral RNA

quantitatively. The HCV primers and probe sequences were selected against the 5′ UTR of the HCV genome. This study used the 4th WHO International Standard for HCV, NIBSC code 06/102, as a standard for viral load estimation. Viral load was expressed as log10 international units per millilitre (log10 IU/ml) [17].

HCV genotyping and subtyping study

HCV genotyping was performed on HCV-RNA-positive patients by amplifying the viral genome's partial core (405 bp) and NS5B (389 bp) gene using nested RT-PCR. The nested PCR reactions and conditions were set as described elsewhere [18]. The PCR amplicons of core (405 bp) and NS5B (389 bp) were visualized in a 1.5% agarose gel using a gel documentation system (Bio-Rad, USA). The positive amplified partial core (405 bp) and NS5B (389 bp) PCR products were gel excised and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) and used for sequencing using Big Dye Terminator 3.1 kit (Applied Biosystem, Foster City, USA) in an automated DNA sequencer 3730 XL (ABI, USA). Sequences were aligned and edited using the Bio-Edit tool. National Center for Biotechnology Information (NCBI) genotyping tool (www.ncbi.nlm.nih.gov/projects/genotyping) was selected for HCV genotypes and subtype determination [18].

Phylogenetic analysis of isolated HCV strain

The phylogenetic analysis used 116 and 113 representatives of partial core and NS5B sequences, respectively. To investigate the evolutionary linkage among laboratory isolates and reference strains. Partial core (351 bp) and NS5B (321 bp) sequences of the representative HCV sequences were aligned with HCV reference strains using the Molecular Evolutionary Genetics Analysis (MEGA-X) software [19]. The evolutionary history was inferred using the Maximum Likelihood method and Kimura 2-parameter model (according to MEGA-X's in-built model selection option) [20]. The tree with the highest log likelihood was constructed for core (-3110.59) and NS5B (-3254.33), respectively. The initial tree for the heuristic search was obtained automatically by applying Neighbor-Join (NJ) and Bio NJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites for core [5 categories (+G, parameter = 0.4677)] and NS5B [5 categories (+G, parameter = 1.8017)], respectively. All positions containing gaps and missing data were eliminated (complete deletion option). The final data set had 360 and 458 positions for the core and NS5B, respectively. Ninety partial core sequences were submitted in the Gen Bank, i.e., 1c (n = 63, MN590016-MN590019, MN642002-MN642058, OR167615-OR167619), 1a (n = 10, MN650197-MN650202, OR167611-OR167612), 1b (n = 2, MN889529-30), 4a (n = 8, MN590020-MN590027), 3b (n = 2, OK148442-43), 3a (n = 5, MN889526-MN889528, OR167620-OR167621) while eighty-nine partial NS5B sequences were submitted in the Gen Bank, i.e., 1c (n = 62, PV232352-PV232414), 1a (n = 10, PV232335-PV232344), 1b (n = 2, PV232333-PV232334), 4a (n = 8, PV239584-239591), 3b (n = 2, PV232345-46), 3a (n = 5, PV232347-PV232351).

Statistical analysis

Categorical demographic data and genotype distribution of patients were compared with the Chi-square test using GraphPad Prism v 9.5.1, CA, USA. Fisher's Exact test was performed for the categorical genotype-based efficacy comparison. A p-value ≤ 0.05 was considered statistically significant. R version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria) was used to generate graph plots.

Results

Demographic data of HCV sero-reactive individuals

A total of 310 HCV sero-reactive CKD individuals were enrolled, of which 50.64% (n = 157) had an active HCV infection (HCV RNA positive). HCV viremia of male patients (51.90%) was slightly higher than that of female patients (48.10%), although no statistical significance was found (p-value = 0.55). Patients between the age group 65–75 years had significantly higher HCV viremia (75%) than patients of any of the other age groups (p-value = 0.001). Additionally, patients who had undergone a higher frequency of dialysis per month (8–12 times/ month) were at a higher risk of HCV infection (p-value = 0.009). Also, patients who lived in the suburban areas had more active HCV infection (59.15%) than patients who lived in rural or urban areas (p-value = 0.95) (Table 1).

HCV genotype distribution

The partial core gene (405 bp) and NS5B (389 bp) of 157 HCV RNA-positive CKD patients were amplified for HCV genotyping. The NCBI genotyping tool (www.ncbi.nlm.nih.gov/projects/genotyping/) was used for genotype determination. Sequence alignment with reference HCV strains revealed that genotype 1 (82.17%, n = 129) was the predominant circulating genotype, followed by genotype 3 (10.19%, n = 16) and genotype 4 (7.64%, n = 12) (Table 2 and Fig. 1a).Six subtypes circulated among this high-risk group, including 1c (67.52%, n = 106), 1a (10.83%, n = 17), 4a (7.64%, n = 12), 3a (6.37%; n = 10), 1b (3.82%, n = 6), and 3b (3.82%, n = 6) (Fig. 1a). It was observed that HCV

Table 1 Comparative analysis of viremia between various demographic patient groups by Chi-square test

Variables		HCV RNA positive (*n* = 157)	Total patients (*N* = 310)	Viremia rate (%) (50.64%)	*p*-Value
Gender	Male	109	210	51.9	0.55
	Female	48	100	48	
Age group (year)	14–24	13	35	37.14	0.001#
	25–34	29	84	34.52	
	35–44	40	77	51.95	
	45–54	42	67	62.69	
	55–64	30	43	69.77	
	65–74	3	4	75	
Dialysis interval	2 times/ month	18	56	31.57	0.009#
	4 times / month	30	72	41.66	
	8 times/ month	109	182	59.89	
Locality of residence	Rural	49	69	41.53	0.95
	Sub-Urban	42	29	59.15	
	Urban	66	55	54.55	

# denotes statistically significant

Gen-1c was the most widespread circulating strain in every variable among CKD patients (Table 2).

Phylogenetic tree construction of isolated HCV strains

The phylogenetic tree was constructed using 116 and 113 partial core and NS5B gene sequences to understand the evolutionary relationship among the HCV strains identified and sequenced during this study. HCV subtype 1c was the significant circulating strain in this CKD study population, in which partial 1c core sequences were clustered to the reference sequences D14853 (Indonesia), AY051292 (India), and KC844047 (China). Moreover the partial 1c NS5B sequences were clustered to reference sequences D14853 (Indonesia), AY651061 (India), and KJ439767 (Canada) (Fig. 1b [A] and [B]).

DAAs efficacy among HCV RNA-positive CKD patients

In this study population, 157 (50.64%) patients were HCV RNA positive, and of these, 141 patients (89.81%) completed DAAs treatment. Among the 141 patients, 66 (46.81%) individuals who completed DAAs treatment were enrolled between January 2017 and December 2018,

Table 2 HCV genotype distribution among various variable groups of CKD patients by Chi-square test

Variables		HCV genotypes						Total (*n* = 157)	p-value
		Genotype 1a (*n* = 17)	Genotype 1b (*n* = 6)	Genotype 1c (*n* = 106)	Genotype 3a (*n* = 10)	Genotype 3b (*n* = 6)	Genotype 4a (*n* = 12)		
Gender	Male	12 (11%)	4 (3.67%)	72 (66.05%)	8 (7.34%)	5 (4.59%)	8 (7.34%)	109 (69.43%)	0.32
	Female	5 (10.42%)	2 (4.17%)	34 (70.83%)	2 (4.17%)	1 (2.08%)	4 (8.33%)	48 (30.57%)	
Age-group (Year)	14–24	1 (7.69%)	1 (7.69%)	8 (61.54%)	1 (7.69%)	1 (7.69%)	1 (7.69%)	13 (8.28%)	0.94
	25–34	5 (17.24%)	1 (3.45%)	19 (65.52%)	1 (3.45%)	1 (3.45%)	2 (6.9%)	29 (18.47%)	
	35–44	3 (7.5%)	2 (5%)	26 (65%)	3 (7.5%)	1 (2.5%)	5 (12.5%)	40 (25.48%)	
	45–54	2 (4.76%)	NIL	33 (78.57%)	4 (9.52%)	1 (2.38%)	2 (4.76%)	42 (26.75%)	
	55–64	5 (16.67%)	2 (6.67%)	18 (60%)	1 (3.33%)	2 (6.67%)	2 (6.67%)	30 (19.11%)	
	65–74	1 (33.33%)	NIL	2 (66.67%)	NIL	NIL	NIL	3 (1.91%)	
Dialysis Interval (n Times/Month)	2 times	2 (16.67%)	1 (8.33%)	5 (41.66%)	1 (8.33%)	1 (8.33%)	2 (16.67%)	12 (7.64%)	0.32
	4 times	4 (18.18%)	1 (4.55%)	13 (59.09%)	1 (4.55%)	1 (4.55%)	2 (9.09%)	22 (14.01%)	
	8 times	6 (6.25%)	2 (2.08%)	75 (78.13%)	5 (5.21%)	2 (2.08%)	6 (6.25%)	96 (61.14%)	
	12 times	5 (18.52%)	2 (7.41%)	13 (48.15%)	3 (11.11%)	2 (7.41%)	2 (7.41%)	27 (17.2%)	
Locality of Residence	Rural	6 (12.24%)	2 (4.08%)	31 (63.27%)	4 (8.16%)	1 (2.04%)	5 (10.20%)	49 (31.21%)	0.49
	sub-Urban	7 (16.67%)	2 (4.76%)	25 (59.52%)	2 (4.76%)	1 (2.38%)	5 (11.90%)	42 (26.75%)	
	Urban	4 (6.06%)	2 (3.03%)	50 (75.76%)	4 (6.06%)	4 (6.06%)	2 (3.03%)	66 (42.03%)	

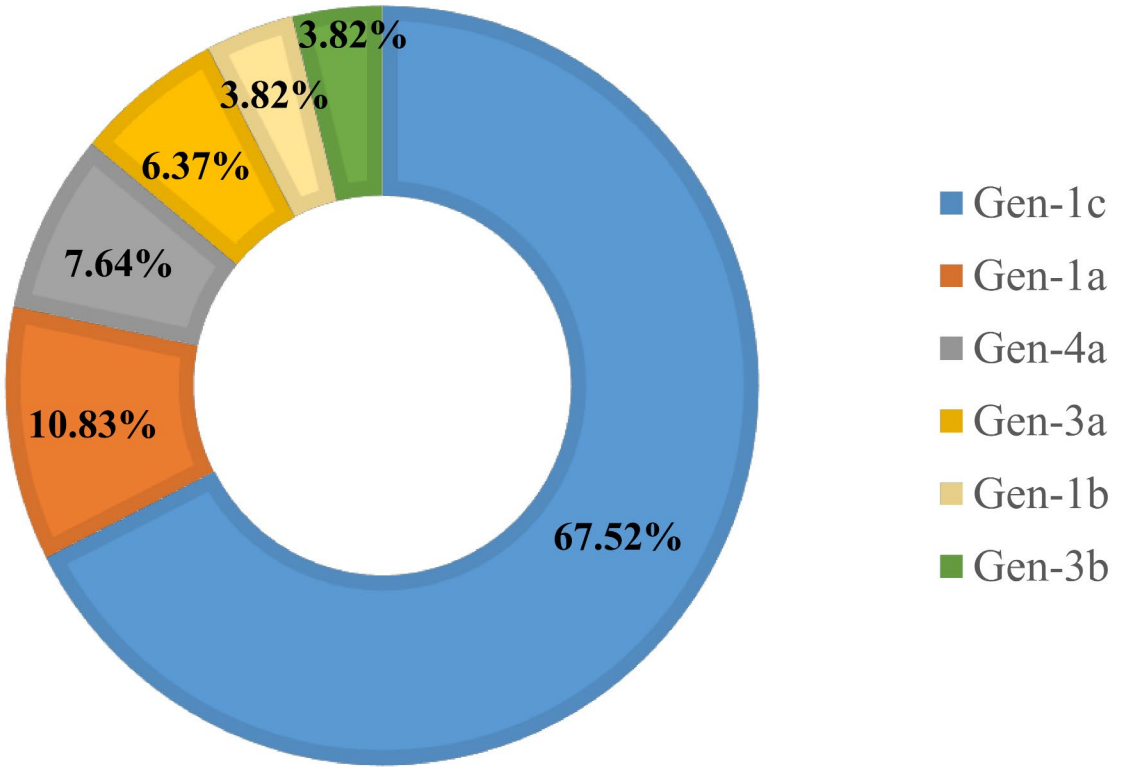

Fig. 1a A donut pie chart shows the distribution of HCV genotypes in CKD patients.

and 75 (53.19%) individuals who completed DAAs treatment were enrolled between January 2019 and December 2021, respectively. Between January 2017 and December 2018, among the 66 patients 66.66% ($n=44$) were prescribed a combination of Sofosbuvir (SOF) and Ledipasvir (LDV), 33.33% ($n=22$) were prescribed a combination of Sofosbuvir (SOF) and Daclatasvir (DCV) whereas, in between January 2019 and December 2021, out of 75 patients, 80% ($n=60$) patients were treated with SOF/DCV, while the rest were treated with SOF/VEL (20%; $n=15$) (Fig. 2a & Table 3).

Moreover, from January 2017 to December 2018, the sustained virologic response (SVR_{12}) efficacy of SOF/LDV was 95.45%, while the SVR_{12} efficacy of SOF/DCV was 100% against CKD patients, and two patients did not attain SVR_{12} with the SOF/LDV treatment (Table 3). During January 2019 to December 2021, the efficacy of SOF/DCV and SOF/VEL was 93.33% and 100%, respectively, and four patients in this period did not attain SVR_{12} in the SOF/DCV treatment (Table 3).

Additionally, the patient who underwent DAAs treatment study revealed that between January 2017 and December 2018, 72.72% ($n=48$) were infected with GT-1c, followed by 1a ($n=6$, 9.09%), 4a ($n=4$, 6.06%), 3a ($n=4$, 6.06%),1b ($n=2$, 3.03%) and 3b ($n=2$, 3.03%) whereas, between January 2019 and December 2021 73.33% ($n=55$) had GT-1c infection followed by 1a ($n=7$, 9.09%), 4a ($n=5$, 6.66%), 3a ($n=4$, 5.33%),1b ($n=2$, 2.66%) and 3b ($n=2$, 2.66%) (Fig. 2b).

Moreover, it was also noticed that the SVR_{12} achievement among HCV GT-1c-infected patients under DAAs treatment during these two phases was 95.83% (46 out of 48) and 92.72% (51 out of 55), respectively (Table 4). In the later phase (January 2019 to December 2021), the SVR_{12} achievement of SOF/DCV reduced to 90.70% from 100% against HCV GT-1c (p-value = 0.55). However, other combinations of DAAs achieved 100% SVR_{12} against other HCV subtypes in both phases (Table 4). The overall DAAs efficacy rate among CKD patients was ˜ 97% and ~95%, respectively, between the two periods (Table 4).

Discussion

The incidence of HCV infection among CKD patients is frequent. However, implementing the International Initiative of Kidney Disease: Improving Global Outcomes (KDIGO) protocol helped reduce HCV infection prevalence among CKD patients in developed countries [21]. However, clinical and therapeutic management of HCV among CKD patients is challenging, especially in developing and low-income countries like India. Therefore, in this five-year (January 2017 to December 2021) study, we

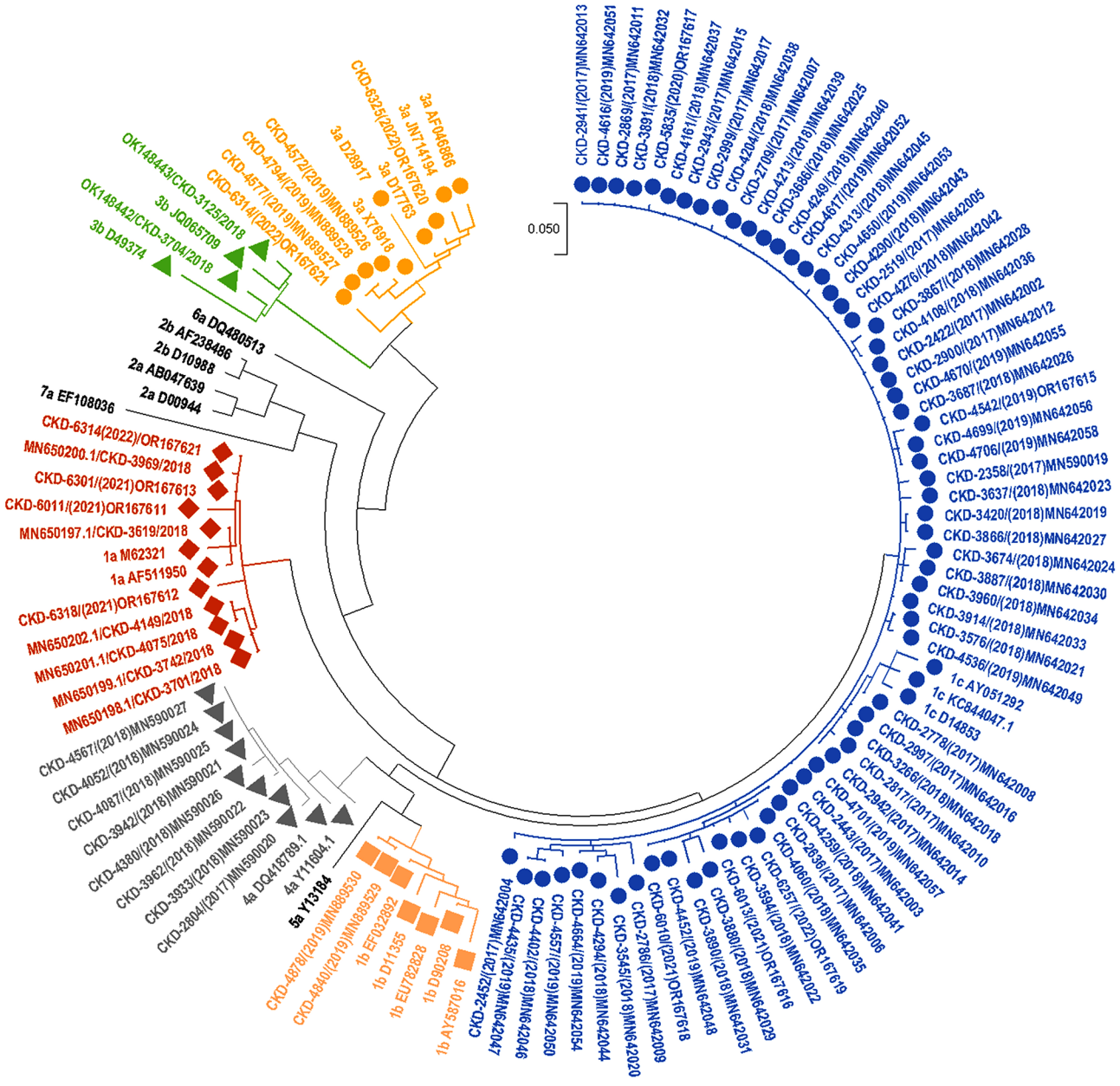

Fig. 1b [**A**]: A phylogenetic tree of partial core sequences of all HCV genotypes isolated among CKD patients

aimed to evaluate the HCV viremia rate, demographic analysis, genomic diversity, and its impact on the DAAs efficacy among CKD patients in West Bengal, India.

In this study, 310 HCV sero-reactive CKD patient samples were referred from different hospitals for routine check-ups to determine the presence or absence of viral RNA. HCV RNA positivity was 50.64% among the sero-reactive population in this study population, indicating the need for intervention in eliminating hospital-acquired HCV infection among CKD patients in this region. It was also observed that CKD patients in sub-urban areas had more active HCV infection than those in urban areas (Table 1). Previous studies reported that HCV infection among patients with CKD varies from 4.3 to 45% in India, corroborating the study data [5–7]. The acquisition of such widespread HCV RNA infection among CKD patients may be due to a lack of awareness about proper HCV screening (maybe testing done during the "window period"), mishandling of contaminated medical equipment during dialysis, number of blood transfusions, nosocomial infections, dialysis treatment duration or cross-contamination of dialysis machines. However, the exact reason for HCV transmission remains unknown among CKD patients [5, 22]. Therefore, each CKD patient and hospital staff should strictly follow the standard infection control guidelines, and each CKD

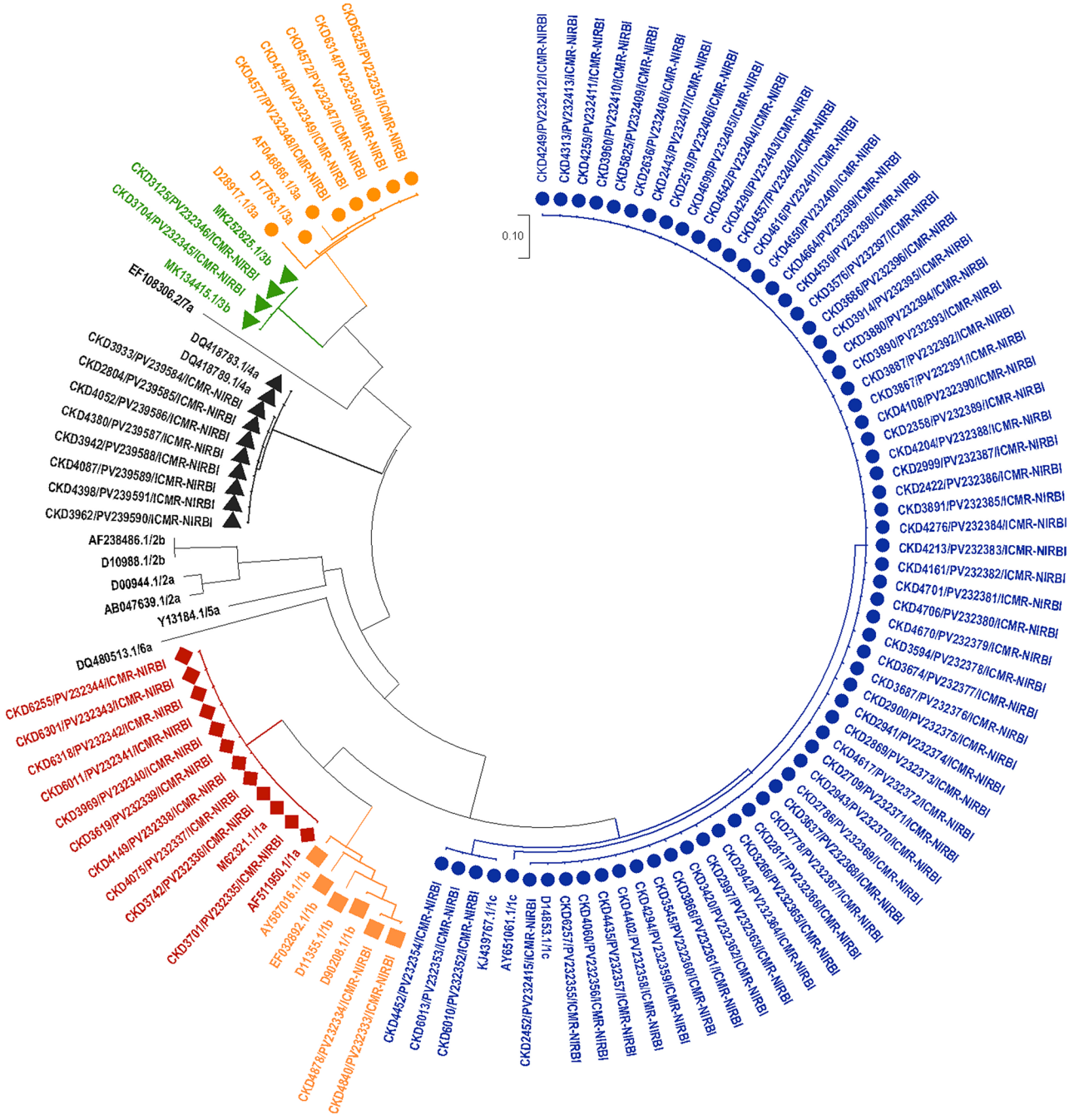

Fig. 1b [**B**]: A phylogenetic tree of partial NS5B sequences of all HCV genotypes isolated among CKD patients

patient should be tested for HCV infection through sensitive NAT-based molecular screening along with ELISA [23]. This study revealed that older patients were more prone to HCV infection (p-value = 0.001) as compared to younger patients. Patients aged 55 to 74 had a higher percentage of HCV RNA positivity than patients of other age groups (Table 1). The reason behind the increased risk of obtaining HCV infection in older CKD patients may be attributed to a weak immune system and the higher chance of spontaneous clearance of HCV among young adults [24, 25]. Patients who undergo frequent dialysis (8 times/month) are more prone to HCV infection (p-value = 0.009) (Table 1). This study's data suggest that more frequent exposure to dialysis settings and hospital environments by CKD patients increases the risk of HCV infection. This finding corroborates a previous study [26].

A few global studies reported the circulation of "unusual" HCV subtypes among HCV-infected patients.

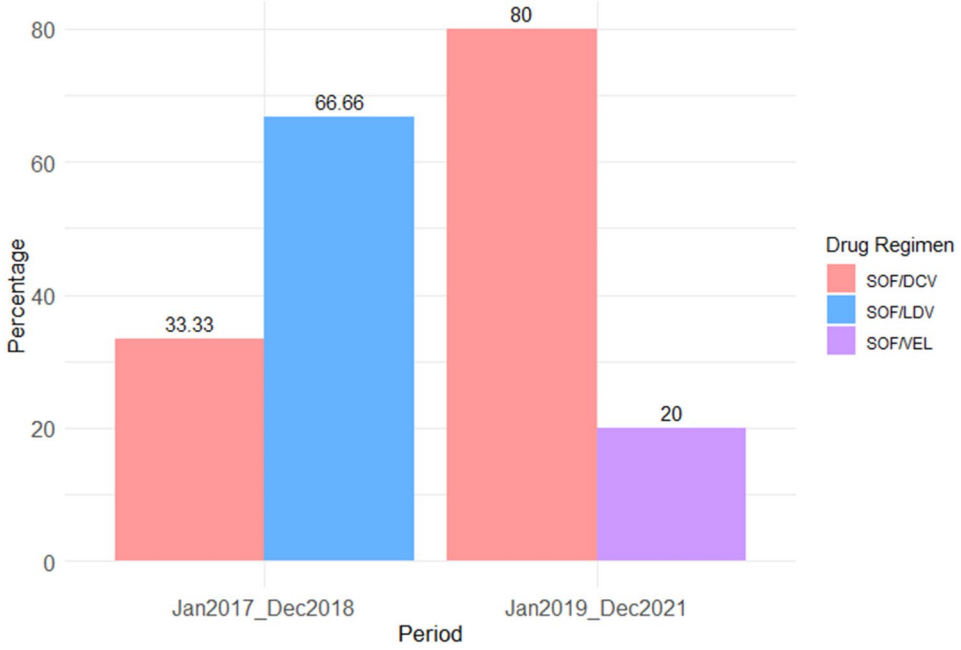

Fig. 2a The distribution of direct-acting antivirals (DAAs) treatment recommended from 2017 to 2021 among CKD patients

Table 3 Treatment efficacy of different combinations of DAAs among CKD patients from 2017 to 2021

Different treatment regimes	The total number of patients treated with DAAs between Jan 2017 and Dec 2021 (*N* = 141)			
	Number of patients treated with DAAs between Jan 2017 and Dec 2018 (*n* = 66)		Number of patients treated with DAAs between Jan 2019 and Dec 2021 (*n* = 75)	
	Number of treated patients (*n*)	SVR_{12} attained (*n*;%)	Number of treated patients (*n*)	SVR_{12} attained (*n*;%)
SOF/DCV	22	22 (100%)	60	56 (93.33%)
SOF/LDV	44	42 (95.45%)	NIL	NIL
SOF/VEL	NIL	NIL	15	15 (100%)

SOF = Sofosbuvir; DCV = Daclatasvir; LDV = Ledipasvir; VEL = Velpatasvir

A study conveyed the prevalence of two uncommon subtypes, 2i (5.6%) and 4d (8.9%), among the Tunisian population [27]. The circulation of "unusual" HCV 1 (47%) and 4 (13.1%) subtypes among the African population was also reported [28, 29]. Another uncommon HCV subtype, 6xg (12%), has been reported among injection drug users (IDU) patients in Myanmar [30]. Even Kalita et al. reported the presence of an atypical HCV subtype 3f (57%) among dialysis patients in northeast India [13]. However, the exact global and local prevalence of atypical HCV subtypes is still poorly understood due to insufficient data in numerous countries across Asia and Africa [31]. Therefore, one of the most significant findings of this study was the highest prevalence of the unusual subtype 1c (67.52%) among this high-risk group population (Table 2) (Fig. 1a). The emergence of the atypical strains may be linked to a common source of infection that may be spread and transmitted through contaminated dialysis equipment or due to CKD patients hopping from one dialysis unit to another [13]. Moreover, in West Bengal, the predominant circulating strain is GT-3a, followed by GT-3b among the general population, whereas among β-thalassemia patients, GT-3a is the prevalent genotype, followed by GT-1b [32, 33]. Additionally, an increase in HCV GT-4a (7.64%) infected cases was observed, which was also unexpected in eastern India. To the best of our knowledge, this is probably the first report on the circulation of two HCV subtypes (1c and 4a) among the HCV-infected population in this region. Therefore, this study demonstrated that the general circulation pattern of the HCV genotypes and subtypes among CKD patients was unusual compared with other community-based HCV genotypes and subtypes prevalence studies in West Bengal.

The real-world DAA's efficacy data against high-risk groups, like CKD, is crucial to eliminating HCV by 2030 [14]. In this study, we observed that between January 2017 and December 2018 and January 2019 and December 2021, the overall DAAs efficacy was ~ 97% and ~ 95%, respectively (Table 4). Briefly, the efficacy of SOF/LDV was approximately 95% between January 2017 and December 2019, and further, no patients were treated with SOF/LDV in the next period (January 2019 and December 2021) (Table 3). The efficacy of SOF/DCV was 100% between January 2017 and December 2018 and its efficacy was reduced to 93.33% between January 2019 and December 2021 (Table 3), while the SOF/VEL

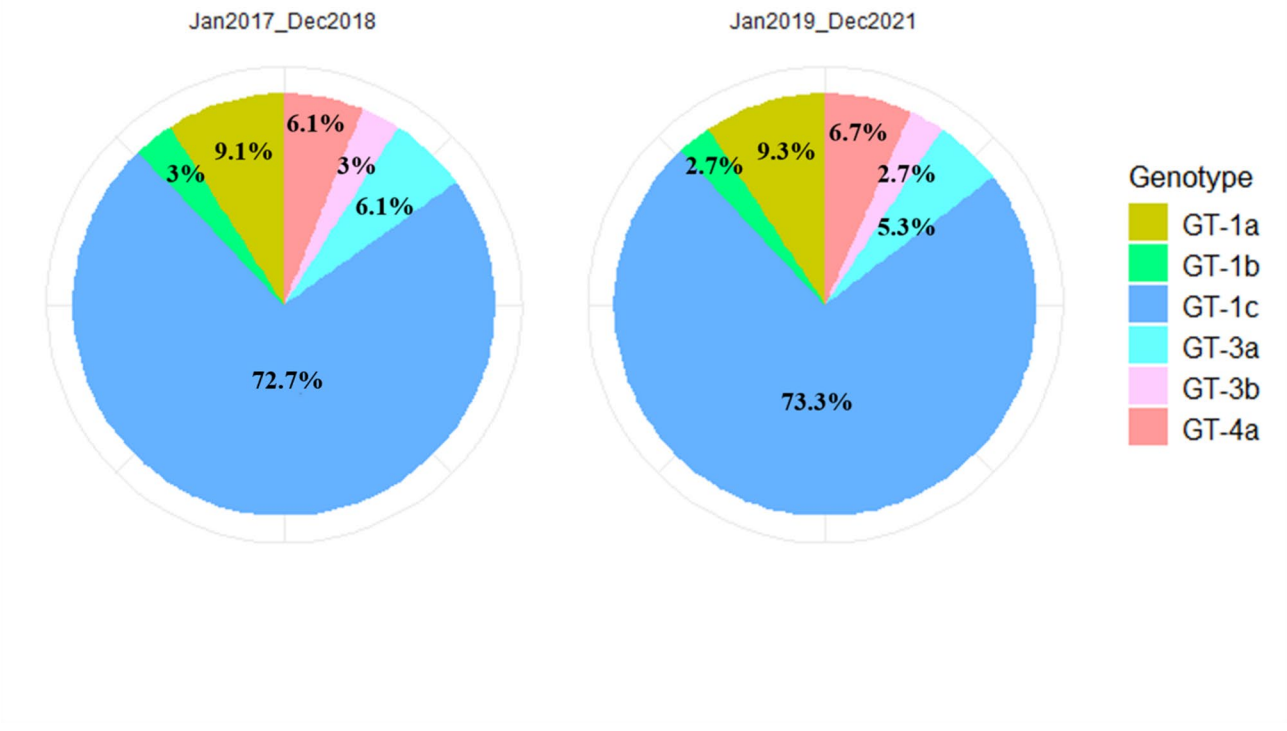

Fig. 2b The comparative analysis of HCV genotype distribution of CKD patients who underwent DAAs treatment

was not recommended from January 2017 to December 2018 and in the next period (January 2019 and December 2021), the efficacy of SOF/VEL remained at 100% (Table 3). Additionally, HCV genotype specific DAAs efficacy study revealed that between January 2017 and December 2018, the efficacy of DAAs against HCV GT-1c infected patients was ~96%, while, between January 2019 and December 2021, the efficacy rate of GT-1c was reduced to ~93%, and the efficacy of other genotype remained at 100% in both periods (Table 4). The reduction in efficacy of DAAs among GT-1c was because of the slight increase in non-responsiveness against SOF/DCV between January 2019 and December 2021. Therefore, it can be concluded that although the reduction of HCV GT-1c efficacy against SOF/DCV was not significant (p-value = 0.55, Table 4), the decrease in SOF/DCV efficacy among GT-1c-infected patients showed the impact in an overall reduction of DAAs efficacy among CKD patients. Afdhal et al. and Pol et al. reported that the SVR_{12} of SOF/LDV and SOF/DCV against HCV GT-1 was 94% and 92%, respectively [34, 35], whereas the SVR_{12} of SOF/VEL against HCV GT-1 was 100% [36]. Thus, this study data substantiates previous studies that SOF/VEL has achieved better SVR_{12} against HCV GT-1 infected patients than SOF/LDV and SOF/DCV. Moreover, according to previous study reports, the DAAs efficacy against unusual HCV GT-1 and GT-4 subtypes was 75% and 56% in the African population [28, 29]. In contrast, European and Asian cohort study data informed the DAAs efficacy against rare HCV subtypes was 94% and 96%, respectively [37, 38]. Therefore, it can be inferred that the DAAs showed better responses against unusual subtypes in our study population than the African population. At the same time, the studied population's DAAs efficacy data were more or less similar to the Asian and European populations. Furthermore, a low prevalence of HCV GT-3b ($n = 6$; 3.82%) was circulating among this high-risk group and achieved 100% SVR_{12}. The HCV GT-3b is a difficult genotype to treat, and a study reported that the achievement of the SVR_{12} rate was 89% in Asia [39]. Thus, it can be inferred that, although the prevalence is low, the DAAs showed optimal response against HCV GT-3b among this high-risk group.

This study certainly has inherent limitations. One of the study's drawbacks was that the baseline and resistance-associated substitutions (RAS) in the DAAs targeted regions (NS5A and NS5B) were not mentioned. Vo-Quang E et al. reported that the NS5A-associated baseline substitutions and RAS-like K24R, M28 V, Q30R, L31M, H58P and A92T are circulating in other unusual DAAs treatment failure HCV GT-1 subtypes (GT-1d,1e and 1 L) patients [40]. Including NS5A and NS5B-associated polymorphism would have strengthened this study. However, our group is working on the RAS analysis of treatment-failure HCV GT-1c patients and other treatment-failed HCV genotype subtypes, such as HCV GT-3a and 3b individuals. Another drawback was that

Table 4 Comparative analysis of treatment efficiency of different HCV genotypes (GTs) from 2017 to 2021 by Fisher's exact test

Treatment Regime	Genotypes	Number of patients who have achieved SVR_{12} against DAAs between Jan 2017 and Dec 2018 (*n* = 64/66; 96.97%)		Number of patients who have achieved SVR_{12} against DAAs between Jan 2019 and Dec 2021 (*n* = 71/75; 94.67%)		p-value
SOF/DCV		SVR_{12} achieved	Non-responder	SVR_{12} achieved	Non-responder	
	Gen-1c	100% (10/10)	0% (0/10)	90.70% (39/43)	9.30% (4/43)	0.55
	Gen-1a	100% (2/2)	0% (0/2)	100% (7/7)	0% (0/7)	-
	Gen-4a	100% (4/4)	0% (0/4)	100% (2/2)	0% (0/2)	-
	Gen-3a	100% (4/4)	0% (0/4)	100% (4/4)	0% (4/4)	-
	Gen-1b	-	-	100% (2/2)	0% (0/2)	-
	Gen-3b	100% (2/2)	0% (0/2)	100% (2/2)	0% (0/2)	-
SOF/LDV	Gen-1c	94.73% (36/38)	5.26% (2/38)	-	-	-
	Gen-1a	100% (4/4)	0% (0/4)	-	-	-
	Gen-4a	-	-	-	-	-
	Gen-3a	-	-	-	-	-
	Gen-1b	100% (2/2)	0% (0/2)	-	-	-
	Gen-3b	-	-	-	-	-
SOF/VEL	Gen-1c	-	-	100% (12/12)	0% (0/12)	-
	Gen-1a	-	-	-	-	-
	Gen-4a	-	-	100% (3/3)	0% (0/3)	-
	Gen-3a	-	-	-	-	-
	Gen-1b	-	-	-	-	-
	Gen-3b	-	-	-	-	-

SOF = Sofosbuvir; DCV = Daclatasvir; LDV = Ledipasvir; VEL = Velpatasvir; Gen = Genotype

this study was conducted during the COVID-19 period. As a result, we faced challenges in tracking the patients' information during the initial phases of the lockdown. However, once the pandemic situation improved, we overcame the obstacle and channelled proper patient connections.

Conclusions

This study is one of the most extensive analyses of the prevalence of the HCV genotype in CKD patients in West Bengal, India. This study highlights the prevalence of circulation of an unusual subtype (HCV GT-1c) among this high-risk group of patients in this region. Therefore, all dialysis centres should strictly follow the national HCV infection control strategy to prevent further HCV infection. Moreover, the efficacy study of DAAs illustrated that although the DAA's efficacy was satisfactory in both phases, the increasing number of SOF/DCV non-responsive cases among CKD patients can be a future concern for the clinical and therapeutic management of HCV. Therefore, a more pan-genotype treatment approach, i.e., SOF/VEL treatment, should be encouraged among this high-risk group.

Abbreviations

HCV	Hepatitis C Virus
CKD	Chronic Kidney Disease
GT	Genotype
DAA	Direct-Acting Antiviral
KDIGO	Kidney Disease: Improving Global Outcome
GFR	Glomerular Filtration Rate
GBD	Global Burden of Disease
HCC	Hepatocellular Carcinoma
WHO	World Health Organization
HRG	High Risk Group
NVHCP	National Viral Hepatitis Programme
SOF	Sofosbuvir
LDV	Ledipasvir
DCV	Daclatasvir
VEL	Velpatasvir
HIV	Human Immunodeficiency Virus
NCBI	National Center for Biotechnology Information
MEGA	Molecular Evolutionary Genetics Analysis
MCL	Maximum Composite Likelihood
NJ	Neighbor-Join
SVR	Sustained Virologic Response
NAT	Nucleic Acid Test
EIA	Enzyme Immunoassay

RIB Ribavirin
RAS Resistance Associated Substitutions

Acknowledgements
We want to acknowledge other Dr. Sadhukhan's laboratory members, especially Miss Maya Halder. We would also like to thank the patients and their families for cooperating and participating in this study.

Author contributions
Conceptualization: P.C.S; Methodology and Lab work: S.B, S.D, A. B and R. D; Software: S.B, S.D, S.N and A.G; Validation: S.B, A.B, S.D, and R.D; Formal Analysis: S.B and S.D; Investigation: P.C.S; Resources: P.C.S; Data Curation: S.B and S.D; Writing Original draft preparation: S.B; Writing– Review & Editing: P.C.S, R.D, S.N, A.G, and U.B; Visualization: S.B; Supervision: P.C.S; Project administration: P.C.S; Funding acquisition: P.C.S.

Funding
The ICMR- National Institute for Research in Bacterial Infections (ICMR-NIRBI, formerly ICMR-NICED) provided all the infrastructure facilities This work was supported by the Department of Science and Technology, Govt. of West Bengal and the Indian Council of Medical Research (ICMR) (grant number 758 (sanc)/ST/P/S&T/9G-8/2014; grant number-VIR/5/2019/ECD-1) respectively.

Data availability
The raw data supporting the conclusions of this article will be made available by the corresponding author upon request.

Declarations

Ethics approval and consent from the participant
The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Indian Council of Medical Research-National Institute of Cholera and Enteric Diseases (ICMR-NICED; recently the name changed to ICMR-NIRBI), Kolkata (Approval number: A-1/2016-IEC; date of approval: 03.10.2016). Informed consent was obtained from all subjects involved in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 27 January 2025 / Accepted: 8 April 2025
Published online: 16 May 2025

References

1. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease Lancet. 2017;389(10075):1238–52. https://doi.org/10.1016/S0140-6736(16)32064-5.
2. Yamagata K, Ishida K, Sairenchi T, Takahashi H, Ohba S, Shiigai T, et al. Risk factors for chronic kidney disease in a community-based population: A 10-year follow-up study. Kidney Int. 2007;71(2):159–66. https://doi.org/10.1038/sj.ki.5002017.
3. Francis A, Harhay MN, Ong ACM, Tummalapalli SL, Ortiz A, Fogo AB, et al. Chronic kidney disease and the global public health agenda: an international consensus. Nat Rev Nephrol. 2024;20(July):473–85. https://doi.org/10.1038/s41581-024-00820-6.
4. Timofte D, Dragos D, Balcangiu-Stroescu A-E, Tanasescu M-D, Balan D, Avino A, et al. Infection with hepatitis C virus in Hemodialysis patients: an overview of the diagnosis and prevention rules within a Hemodialysis center (Review). Exp Ther Med. 2020;109–16. https://doi.org/10.3892/etm.2020.8606.
5. Madhavan A, Sachu A, Balakrishnan AK, Vasudevan A, Balakrishnan S, Vasudevapanicker J. Prevalence of hepatitis C among haemodialysis patients in a tertiary care hospital in South India. Iran J Microbiol. 2020;12(6):644. https://doi.org/10.18502/IJM.V12I6.5041.
6. Agarwal SK, Bagchi S, Yadav RK. Hemodialysis patients treated for hepatitis C using a Sofosbuvir-based regimen. Kidney Int Rep. 2017;2(5):831–5. https://doi.org/10.1016/j.ekir.2017.04.003.
7. Agarwal SK, Dash SC, Irshad M. Hepatitis C virus infection during haemodialysis in India. J Assoc Physicians India. 1999;47(12):1139–43.
8. Shi G, Suzuki T. Molecular basis of encapsidation of hepatitis C virus genome. Front Microbiol. 2018;9(MAR):1–7. https://doi.org/10.3389/fmicb.2018.00396.
9. World Health Organization. Global Hepatitis Report 2024.
10. Ministry of Health and Family Welfare G. National Action Plan Combating Viral Hepatitis in India. 2019;1–52.
11. Hedskog C, Parhy B, Chang S, Zeuzem S, Moreno C, Shafran SD, et al. Identification of 19 novel hepatitis C virus subtypes-further expanding HCV classification. Open Forum Infect Dis. 2019;6. https://doi.org/10.1093/ofid/ofz076.
12. Roy P, Patel A, Lole K, Gupta RM, Kumar A, Hazra S. Prevalence and genotyping pattern of hepatitis C virus among patients on maintenance Hemodialysis at five centers in Pune, India. Med J Armed Forces India. 2019;75(1):74–80.
13. Kalita D, Deka S, Chamuah K. Circulation of an atypical hepatitis C virus (HCV) strain at a dialysis unit in northeast India. Microbiologyopen. 2021;10(1):e1147. https://doi.org/10.1002/mbo3.1147
14. Venkatesh SRKD. Diagnosis & management of viral hepatitis. Natl Heal Mission. 2018;1(1):1–80.
15. Hepatitis C. Getting tested. Atlanta (GA): U.S. Department of Health and Human Services; https://www.cdc.gov/hepatitis/hcv/pdfs/hepcgettingtested.pdf
16. Omata M, Kanda T, Wei L, Yu ML, Chuang WL, Ibrahim A, et al. APASL consensus statements and recommendation on treatment of hepatitis C. Hepatol Int. 2016;10(5):702–26.
17. Firdaus R, Saha K, Sadhukhan PC. Rapid immunoassay alone is insufficient for the detection of hepatitis C virus infection among high-risk population. J Viral Hepat. 2013;20(4):290–3.
18. Saha K, Firdaus R, Biswas A, Mukherjee A, Sadhukhan PC. A novel nested reverse-transcriptase polymerase chain reaction method for rapid hepatitis C virus detection and genotyping. Indian J Med Microbiol. 2014;32(2):130–6.
19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547–9.
20. Kimura M. J Mol Evol ©. 1980;16:1330.
21. Stevens PE, Ahmed SB, Carrero JJ, Foster B, Francis A, Hall RK, et al. KDIGO 2024 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int. 2024;105(4):S117–314.
22. Anand A, Shalimar. Hepatitis C virus in India: challenges and successes. Clin Liver Dis (Hoboken). 2021;18(3):150–4. https://doi.org/10.1002/cld.1137.
23. Yu YC, Wang Y, He CL, Wang MR, Wang YM. Management of hepatitis C virus infection in Hemodialysis patients. World J Hepatol. 2014;6(6):419. https://doi.org/10.4254/WJH.V6.I6.41.
24. Marcus EL, Tur-Kaspa R. Chronic hepatitis C virus infection in older adults. Clin Infect Dis. 2005;41(11):1606–1612. https://doi.org/10.1086/497597
25. Seo S, Silverberg MJ, Hurley LB, et al. Prevalence of spontaneous clearance of hepatitis C virus infection doubled from 1998 to 2017. Clin Gastroenterol Hepatol. 2020;18(2):511–3. https://doi.org/10.1016/j.cgh.2019.04.035.
26. Pawlotsky JM, Negro F, Aghemo A, Berenguer M, Dalgard O, Dusheiko G, et al. EASL recommendations on treatment of hepatitis C: final update of the series☆. J Hepatol. 2020;73(5):1170–218.
27. Khedhiri M, Ghedira K, Rajhi M, Hammemi W, Sadraoui A, Touzi H, et al. Overview of the epidemic history of hepatitis C uncommon subtypes 2i and 4d in Tunisia and in the world. Infect Genet Evol. 2022;105(October):105375. https://doi.org/10.1016/j.meegid.2022.10537.
28. Gupta N, Mbituyumuremyi A, Kabahizi J, Ntaganda F, Muvunyi CM, Shumbusho F, et al. Treatment of chronic hepatitis C virus infection in Rwanda with ledipasvir–sofosbuvir (SHARED): a single-arm trial. Lancet Gastroenterol Hepatol. 2019;4(2):119–26. https://doi.org/10.1016/S2468-1253(18)30382-0.
29. Pawlotsky JM. DAA failures in African patients with unusual HCV subtypes: hey! Didn't you know there was another world? J Hepatol. 2019;71(6):1070–2. https://doi.org/10.1016/j.jhep.2019.09.021.
30. Ye M, Chen X, Wang Y, Duo L, Zhang C, Zheng YT. Identification of a new HCV subtype 6xg among injection drug users in Kachin. Myanmar Front Microbiol. 2019;10:814. https://doi.org/10.3389/fmicb.2019.00814.
31. Vo-Quang, E., & Pawlotsky, J. M.'Unusual' HCV genotype subtypes: origin, distribution, sensitivity to direct-acting antiviral drugs and behaviour on antiviral treatment and retreatment. Gut.2024; 73(9), 1570–1582. https://doi.org/10.1136/gutjnl-2024-332177
32. Chowdhury A, Santra A, Chaudhuri S, Dhali GK, Chaudhuri S, Maity SG, Naik TN, Bhattacharya SK, Mazumder DN. Hepatitis C virus infection in the general

population: a community-based study in West Bengal, India. Hepatology. 2003;37(4):802–9. https://doi.org/10.1053/jhep.2003.50157.
33. Dutta S, Biswas A, Bakshi S, Choudhury P, Das R, Nath S, et al. Molecular epidemiology of HCV infection among Multi-Transfused β-Thalassemia patients in Eastern India: A Six-Year observation. Thalass Rep. 2023;13(3):165–78.
34. Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, et al. Ledipasvir and Sofosbuvir for previously treated HCV genotype 1 infection. N Engl J Med. 2014;370(16):1483–93. https://doi.org/10.1056/nejmoa1316366.
35. Pol S, Bourliere M, Lucier S, Hezode C, Dorival C, Larrey D, et al. Safety and efficacy of daclatasvir-sofosbuvir in HCV genotype 1-mono-infected patients. J Hepatol. 2017;66(1):39–47. https://doi.org/10.1016/j.jhep.2016.08.021.
36. Zignego AL, Monti M, Gragnani L. Sofosbuvir/Velpatasvir for the treatment of hepatitis C virus infection. Acta Biomed. 2018;89(3):321–31. https://doi.org/10.23750/abm.v89i3.7718.
37. Dietz J, Graf C, Berg CP, Port K, Deterding K, Buggisch P et al. Rare HCV subtypes and retreatment outcomes in a cohort of European DAA-experienced patients. JHEP Rep. 2024;6(7).
38. Lim SG, Phyo WW, Shah SR, Win KM, Hamid S, Piratvisuth T, et al. Findings from a large Asian chronic hepatitis C real-life study. J Viral Hepat. 2018;25(12):1533–42.
39. Wei L, Lim SG, Xie Q, Văn KN, Piratvisuth T, Huang Y, et al. Sofosbuvir–velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. Lancet Gastroenterol Hepatol. 2019;4(2):127–34.
40. Vo-Quang E, Soulier A, Ndebi M, Rodriguez C, Chevaliez S, Leroy V et al. Virological characterization of treatment failures and retreatment outcomes in patients infected with unusual HCV genotype 1 subtypes. Vol. 78, Hepatology. 2023. 607–620 p.

Publisher's note